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Cytaeis capitata Puce *et al.*, 2004 (Cnidaria, Hydrozoa) Newly Recorded from Amami-Oshima Island, Southwestern Japan

Hiroshi Namikawa[1] and Yuichi Kameda[2]

[1] Department of Zoology, National Museum of Nature and Science,
4–1–1 Amakubo, Tsukuba, Ibaraki 305–0005, Japan
E-mail: namikawa@kahaku.go.jp
[2] Center for Molecular Biodiversity Research, National Museum of Nature and Science,
4–1–1 Amakubo, Tsukuba, Ibaraki 305–0005, Japan

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Abstract *Cytaeis capitata* Puce *et al.*, 2004, previously known only from the type locality (North Sulawesi, Indonesia) is newly recorded from Amami-Oshima Island, southwestern Japan. Collected during the 2017–18 "KUROSHIO project" of the National Museum of Nature and Science, Tsukuba, the species was identified from a molecular analysis of mitochondrial 16S ribosomal DNA sequences, in addition to a morphological examination. The molecular phylogenetic analysis also revealed a close relationship between *C. capitata* and *C. uchidae* Rees, 1962. All specimens of *C. capitata*, the fifth species of *Cytaeis* recorded from Japan, were found on the shells of the previously-recognized host gastropod species *Nassarius globosus* (Quoy & Gaimard, 1833).

Key words: Amami-Oshima Island, *Cytaeis capitata*, new record, 16S DNA.

Introduction

Hydrozoan species in the genus *Cytaeis* (Cytaeididae) are epizoic, growing on the shells of distinct species of living gastropods (Bouillon *et al.*, 2006). Three *Cytaeis* species, each growing on different gastropod species, have been previously described from Sagami Bay, Japan (Rees, 1962; Uchida, 1964), in addition to a fourth, also living on a different gastropod species, from Tateyama, Chiba, Japan (Namikawa & Deguchi, 2013).

In 2017–2018, an opportunity to further survey *Cytaeis* species in Japan arose during the survey of marine hydrozoa of Amami-Oshima Island, southwestern Japan, being part of the "KUROSHIO project" (Integrated research of geological, biological, and anthropological histories in relation to the Kuroshio Current) conducted from 2016 by the National Museum of Nature and Science, Tsukuba. In this survey, hydrozoan specimens growing on the shells of living *Nassarius globosus* (Quoy & Gaimard, 1833) were collected from Amami-Oshima Island and tentatively identified morphologically as *Cytaeis* sp., due to the host gastropod species having been previously unknown in Japan. Subsequently, the specimens were subjected to molecular analysis (in addition to traditional morphological examination) for specific identification. The phylogenetic relationships among the known Japanese *Cytaeis* species, based on molecular analysis of mitochondrial 16S ribosomal DNA sequences, is also briefly discussed.

Materials and Methods

Five hydrozoan colonies (*Cytaeis* sp.) on the shells of living *Nassarius globosus* (Fig. 1a) were collected from the intertidal zone at Fukaura, Setouchi, Amami-Oshima Island on 5 July 2018 during a faunal survey of marine

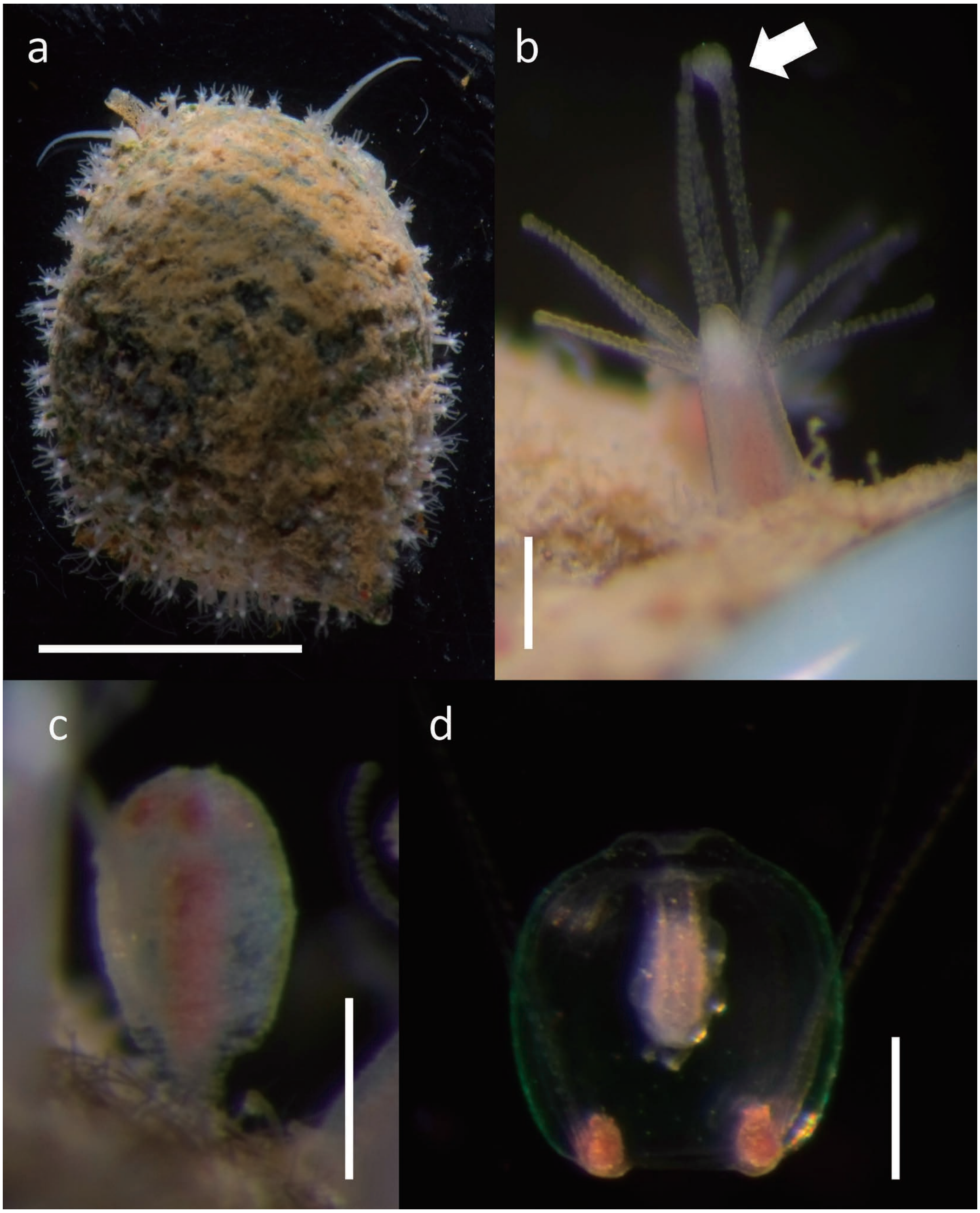


Fig. 1. *Cytaeis* sp. from Amami-Oshima Island. a, hydrozoan colony growing on a shell of *Nassarius globosus*. b, a polyp with a capitate tentacle (indicated by arrow) distributed along the aperture of the host gastropod shell. c, a medusa bud completely covered with periderm. d, 2-day old female medusa after liberation from a hydrozoan colony in the laboratory. Scale = 5 mm (a), 0.5 mm (b–d).

hydrozoa at Amami-Oshima Island, conducted as part of the "KUROSHIO project" of the National Museum of Nature and Science, Tsukuba.

All hydrozoan colonies growing on the collected gastropod shells were conveyed alive to the National Museum of Nature and Science,

Table 1. Measurements (mean ± S.D., range) of *Cytaeis* sp. from Amami-Oshima Island

	P (*n* = 30)	MB (*n* = 30)	NM (*n* = 30)	MM (*n* = 30)
Height of body (mm)	1.3 ± 0.2 (1.0–1.5)	0.8 ± 0.1 (0.6–0.9)	0.7 ± 0.1 (0.5–0.9)	1.0 ± 0.2 (0.8–1.2)
Width of body (mm)	0.2 ± 0.04 (0.2–0.3)	0.3 ± 0.03 (0.3–0.4)	0.7 ± 0.1 (0.5–0.9)	1.0 ± 0.2 (0.8–1.2)
Length of stalk (mm)		0.2 ± 0.1 (0.1–0.3)		
Length of manubrium (mm)			0.4 ± 0.1 (0.2–0.6)	0.8 ± 0.1 (0.6–0.9)
No. of oral tentacles	13 ± 1.4 (10–15)		4	4
No. of marginal tentacles			4	4

P: polyps; MB: medusa buds; NM: medusae within 24 hrs after liberation; MM: medusae with matured gonads 2–3 days after liberation.

Table 2. Dimensions (mean ± S.D., range) of each nematocyst type of *Cytaeis* sp. from Amami-Oshima Island

	n	Length	Width
Polyps			
Desmonemes (tentacles)	30	6.2 ± 0.3 (5.6–6.4)	3.7 ± 0.3 (3.2–4.0)
Microbasic euryteles			
(tentacles)	30	8.5 ± 0.5 (8.0–9.2)	4.1 ± 0.2 (3.6–4.4)
(only capitate tentacles)	30	19.3 ± 0.6 (18.4–20.0)	10.2 ± 0.4 (9.6–10.2)
Medusae			
Desmonemes (marginal tentacles)	30	6.7 ± 0.4 (6.0–7.0)	4.3 ± 0.2 (4.0–4.6)
Microbasic euryteles			
(umbrella and marginal tentacles)	30	6.9 ± 0.1 (6.8–7.0)	4.9 ± 0.1 (4.8–5.0)
(tip of oral tentacles)	30	10.0 ± 0.1 (9.8–10.2)	4.9 ± 0.1 (4.8–5.0)

Tsukuba, and maintained in the laboratory in culture containers (8 cm in diameter, 4 cm in height) filled with artificial seawater (Marine Art SF-1: Osakayakken. Co. Ltd, Osaka) at 23–25°C so as to obtain medusae, vital for distinguishing between hydrozoan species. The hydrozoan colonies on the gastropod shells were fed with nauplius of *Artemia* sp., and their host gastropods, with flake-like fish food, once per week. Seawater in the culture containers was renewed almost daily. The resulting medusae were cultured until maturity under the same conditions as the hydrozoan colonies. Colony and medusae morphologies were observed under a binocular stereo microscope (Table 1), and sizes of each nematocyst type observed in polyps and medusae measured under a biological microscope (Table 2).

For specific identification of the specimens from Amami-Oshima Island and clarification of the phylogenetic relationship among Japanese *Cytaeis* species, a partial region of mitochondrial 16S ribosomal DNA was sequenced, being generally used for DNA barcoding in hydrozoa (Schuchert, 2018). Specimens of four known Japanese *Cytaeis* species (*C. imperialis* Uchida, 1964, *C. kakinumae* Namikawa & Deguchi, 2013, *C. nuda* Rees, 1962 and *C. uchidae* Rees, 1962) in addition to *Cytaeis* sp. from Amami-Oshima Island were newly collected for molecular analysis, with examples of *Solanderia* sp. (collected from Amami-Oshima Island on 9 November 2017) utilized as an outgroup. Total genomic DNA was extracted using NucleoSpin Tissue XS (TaKaRa). A partial region of the 16S gene (about 500–600 bp) was amplified by the polymerase chain reaction (PCR) using the primers SHA (5′-ACGGAATGAACTCAAATCA-TGT-3′) and SHB (5′-TCGACTGTTTACC-

AAAAACATA-3′) (Cunningham & Buss, 1993) and the following reaction condition: initial denaturation for 5 min at 94°C, followed by 30 cycles of 20 s at 94°C, 45 s at 45°C, and 2 min at 68°C, with final extension for 10 min at 68°C. All reactions were aided by ExTaq HS polymerase (TaKaRa), and the products purified by ExoSAP-IT (Thermo Fisher Scientific). Sequencing reactions were performed according to the manufacturer's instructions using the BigDye Terminator Cycle Sequencing Reaction Kit ver. 3.1 (Thermo Fisher Scientific). Labeled fragments were analyzed using an ABI 3500xL Genetic Analyzer (Applied Biosystems). The nucleotide sequences have been submitted to the DNA Data Bank of Japan (DDBJ) under accession numbers LC439497–LC439507.

The obtained sequences and the sequence of *C. capitata* from GenBank (KP776769) were aligned using Muscle method implemented in MEGA7 (Kumar *et al.*, 2016). Phylogenetic trees were obtained by maximum parsimony (MP) and maximum likelihood (ML) methods implemented in MEGA. Prior to the analyses, we determined an appropriate model of sequence evolution and model parameters using MEGA. Based on the selected model (GTR + I + G), MP and ML analyses were performed with heuristic searches with subtree-pruning-regrafting-extensive (SPR) branch swapping. Nodal support for the MP and ML analyses were assessed using bootstrap method with 1000 replications.

Results and Discussion

Measurements of the Amami-Oshima Island *Cytaeis* sp. colonies and medusae (shown in Table 1) were consistent with those of *C. capitata* described by Puce *et al.* (2004). The nematocyst composition of *Cytaeis* sp. also agreed with that of *C. capitata* (Table 2), with further correspondence with *C. capitata* in the following characters; host gastropod species (*Nassarius globosus* = *Pliarcularia globosus* [sic] in Puce *et al.*, 2004) (Fig. 1a), the polyps having capitate tentacles distributed along the aperture of the host shell (Fig. 1b), and the fusiform medusa-buds completely covered by periderm (Fig. 1c).

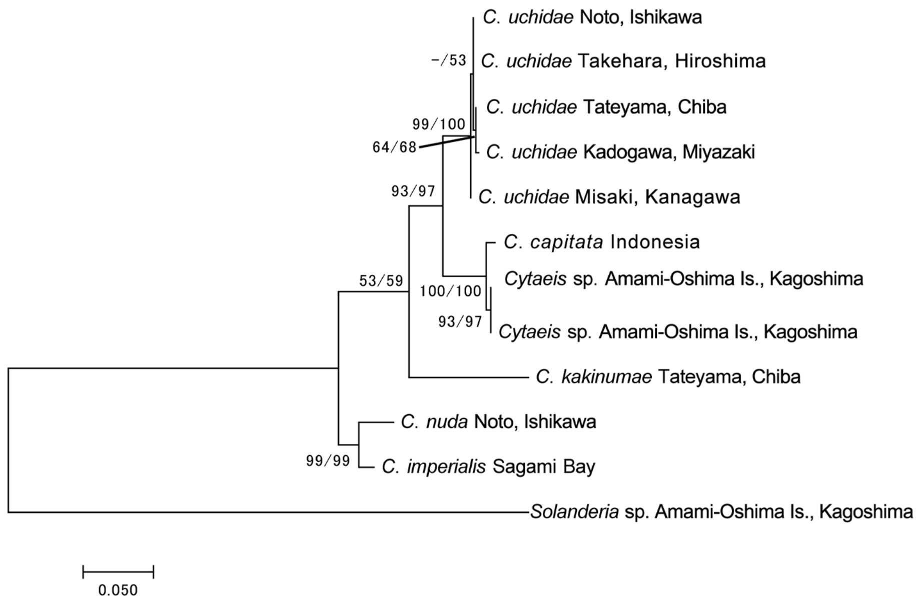


Fig. 2. Maximum likelihood tree showing phylogenetic relationships among *Cytaeis* species from Japan. The tree was based on 584 bp of mitochondrial 16S rDNA sequences (-ln likelihood = 1732.2382). Numbers on branches indicate bootstrap values for MP and ML analyses (shown only for higher nodes with > 50).

The molecular phylogenetic analyses also recovered *C. capitata* from Indonesia and *C.* sp. from Amami-Oshima Island as monophyletic with low genetic divergence (0.9%; Fig. 2), suggesting that they are closely related. Accordingly, the Amami-Oshima specimens were identified as *C. capitata*. Amami-Oshima Island is the only known locality of the species apart from the type locality (North Sulawesi, Indonesia). This finding suggests that *C. capitata* may be widespread across the tropical Indo-Pacific region, where the host gastropod occurs. On this basis, Amami-Oshima Island is considered to be the northern limit of both species (see Tsuchiya, 2017).

Because *C. capitata* resembles *C. uchidae* in medusa morphology (Fig. 1d), it is possible that the *Cytaeis* medusae reported from Amami-Oshima Island by Kubota (2006) were of *C. capitata*, although identified at the time as *C. uchidae*. Polyps with capitate tentacles have also been found in *C. uchidae* colonies, distributed along the aperture of the host gastropod *Nassarius livescence* (Philippi, 1849) (Namikawa, 2005). A close relationship between the two species was strongly supported by the molecular phylogenetic analyses (Fig. 2).

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